Protocol for genotyping LE-Rosa26Tm1(LSL-Cas9)Ottc transgenic rats
January 16, 2019

Genomic DNA Preparation by Macherey-Nagel Tissue Spin Columns

Using this kit according to the manufacturer's protocol is the preferred way for preparing genomic DNA at OTTC when it is intended for ddPCR (i.e. copy-number quantification). Typically, 10 to 90 ng of genomic DNA are used in a 25uL PCR reaction.

General PCR reaction setup:

12.0 uL 2x Q5 master mix (New England Biolabs)
12.0 uL 2x specific oligos (1 uM Forward + 1uM Reverse; in water)
1.0 uL genomic DNA
25.0 uL PCR reaction

PCR Program CR1943		
Line	**Temp**	**Time**
Step 1	98oC	HOLD (hot start)
Step 2	98oC	30 sec
Step 3	98oC	10 sec
Step 4	60oC	30 sec
Step 5	72oC	2 min
Step 6	Go to Step 2	Repeat 34x
Step 7	72oC	5 min
Step 8	12oC	HOLD

Primer Sequences

Primer Name	**Primer Sequence (5' … 3')**	**Amplicon**
Cas9 F4889	CAGGCCGAGAATATCATCCACC	3' junction
Rosa26 R67001	TTCTGCATTCCAGAAGGAACTAACTTTTATAGAG	3' junction

3' junction

These oligos (Cas9 F4889 and Rosa26 R67001) produce a 1600 bp amplicon and work with Q5 polymerase and program CR1943. This assay is not suitable for the detection of the LSL-nickase rat that was also developed by NIDA/OTTC.

This protocol was updated on 01-16-2019 by CR.
Any questions regarding protocol, contact nidatransgenicprojects@mail.nih.gov.